

## CARDIOVASCULAR MEDICINE

# Raised concentrations of macrophage colony stimulating factor in severe unstable angina beyond the acute phase are strongly predictive of long term outcome

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**Objective:** To evaluate the long term prognostic value of macrophage colony stimulating factor (MCSF), interleukin-6 (IL-6), and tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) measured in serum six weeks after the occurrence of unstable angina.

**Subjects:** 119 consecutive patients, mean (SD) age 58 (10) years, with severe unstable angina (Braunwald class IIIb); controls were 96 subjects of similar age and sex distribution.

**Design:** MCSF, IL-6, and TNF $\alpha$  were measured on admission, at discharge, and six weeks later, and the patients were followed for two years. Clinical end points were: cardiac death, readmission for acute coronary syndromes, and revascularisation.

**Setting:** District general hospital.

**Results:** 113 patients completed follow up, during which two died of non-cardiac causes. Of the remaining 111 patients, 39 (35.1%) had a cardiac event (two deaths, 15 revascularisations, and 22 readmissions for acute coronary syndromes). MCSF and IL-6 concentrations at six weeks were higher in patients with cardiac events than in those without ( $424 \pm 306$  pg/ml,  $p = 0.0008$ , and  $6.6 \pm 4.5$  pg/ml,  $p = 0.01$ , respectively). Cytokine concentrations at six weeks were also significantly higher than in the control group. Logistic regression analysis showed that MCSF concentrations were the only independent predictors of future events, with an adjusted odds ratio for events of 4.1 (95% confidence interval 1.1 to 14.8;  $p = 0.03$ ). The two year survival free of cardiac events was significantly lower in patients with MCSF concentrations in the highest tertile (values  $\geq 468$  pg/ml) than in those with values  $< 468$  pg/ml.

**Conclusions:** Increased MCSF concentrations beyond the acute phase are strongly predictive of long term outcome in patients with severe unstable angina.

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It is becoming increasingly clear that inflammation plays a central role in the pathogenesis of acute coronary syndromes.<sup>1,2</sup> A rise of the acute phase proteins, such as C reactive protein, is a strong predictor of short and long term prognosis in unstable angina.<sup>3,4</sup> There are few data on the long term prognostic value of proinflammatory cytokines in unstable angina. High macrophage colony stimulating factor (MCSF) concentrations were independent predictors of coronary events in patients with angina during a 14 month follow up.<sup>5</sup> In another study,<sup>6</sup> raised admission values of interleukin-6 (IL-6) and tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) were strong predictors of coronary events in patients with unstable angina during a 17 month follow up. However, the current easy access to revascularisation procedures, mainly early percutaneous coronary interventions (PCI), may change the natural history of coronary artery disease, as many patients are now treated invasively.<sup>4</sup> Thus inflammatory markers during the acute phase may have a reduced prognostic value in the long term. The present study was conducted to explore the long term prognostic value of MCSF, IL-6, and TNF $\alpha$  measured six weeks after the occurrence of unstable angina.

## METHODS

### Patients

We studied 119 consecutive patients (94 men and 25 women), mean (SD) age 58 (10) years (range 38-75 years), who were admitted to our coronary care unit with severe unstable angina (Braunwald class IIIb). Exclusion criteria were recent myocardial infarction (within the last three months), coronary artery bypass graft (CABG), clinical

evidence of heart failure, age more than 75 years, and co-existent neoplasia or inflammatory disease.

MCSF, IL-6, and TNF $\alpha$  concentrations were determined in serum on admission and at discharge. Six weeks after discharge all patients were invited to reattend our clinic for repeat cytokine and lipid measurements. Initially, 130 patients were recruited during their hospital admission. However, six weeks after discharge 119 patients attended the appointment for blood sampling and these formed the study group. Follow up information was obtained from outpatient visits (every three months) or by telephone interview with the patient or the family doctor. Six patients could not be contacted or were unwilling to cooperate. Thus 113 patients completed the two year follow up.

The start of the follow up period was the day of blood sampling (41 (7) days after discharge) and the mean follow up period was 23 (7) months. Clinical end points were cardiac death, readmission for acute coronary syndromes, and revascularisation with PCI or CABG because of new or worsening symptoms. Only the first event was counted as an end point.

Ninety six subjects without coronary artery disease or diabetes mellitus, matched for age and sex, served as a healthy control group.

**Abbreviations:** CABG, coronary artery bypass graft; IL-6, interleukin-6; MCSF, macrophage colony stimulating factor; PCI, percutaneous coronary intervention; TNF $\alpha$ , tumour necrosis factor  $\alpha$

### Laboratory methods

MCSF was assayed by a quantitative sandwich immunoassay technique (R&D Systems Europe, Abingdon, UK) with a range from 31.2–2000 pg/ml. IL-6 and TNF $\alpha$  were assayed by quantitative high sensitivity sandwich immunoassay techniques (R&D Systems) with ranges from 0.156–10.0 pg/ml and from 0.5–32.0 pg/ml, respectively. The intra-assay and inter-assay coefficient of variation was < 5% for MCSF measurements, < 11% for TNF $\alpha$ , and < 10% for IL-6. All measurements were done in duplicate.

### Statistical analysis

The data on MCSF, IL-6, and TNF $\alpha$ , which were not normally distributed, are expressed as medians. Differences between and within groups were analysed by the Wilcoxon signed rank test or the Mann-Whitney U test as appropriate. For repeated measurements, comparisons between groups were carried out with Freedman analysis of variance (ANOVA); for a probability value of  $p < 0.05$ , pairwise comparisons were done with the Wilcoxon test using Bonferroni's correction. Discontinuous variables were tested by a contingency  $\chi^2$  test. Spearman's rank correlation test was used to assess relations between variables. In order to evaluate the independent contribution of MCSF, IL-6, and TNF $\alpha$  to the risk of events during follow up, logistic regression analysis was used, with age, sex, hypertension, and diabetes mellitus as possible confounding factors. In this model, logarithmic transformation was made on MCSF, IL-6, and TNF $\alpha$  concentrations. Event-free survival was analysed by the Kaplan-Meier method, and the log-rank test was used for comparison between curves. A probability value of  $p < 0.05$  was considered significant. The STATISTICA 2002 version 6 statistical package was used (StatSoft, Tulsa, Oklahoma).

### RESULTS

Baseline characteristics of the patients studied are summarised in table 1. Patients with cardiac events during follow up were older than those without events. In addition, patients with events were more often hypertensive and diabetic, but these differences did not reach significance. The two subgroups did not differ with respect to the reported use of aspirin (87.2% *v* 90.3%,  $p = 0.8$ ),  $\beta$  blockers (74.4% *v* 73.6%,  $p = 0.9$ ), angiotensin converting enzyme inhibitors (30.8% *v* 27.8%,  $p = 0.9$ ), or statins (38.5% *v* 34.7%,  $p = 0.8$ ) at the end of the study.

### Cardiac events

During their hospital admission, 23 patients underwent revascularisation (22 PCI and one CABG). Within six weeks

of discharge, 11 patients were revascularised electively (six PCI and five CABG). Of the 111 patients who completed the two year follow up, 39 (35.1%) had a cardiac event: two died, 15 had a revascularisation procedure (six CABG and nine PCI) because of clinical deterioration, and 22 were re-admitted for acute coronary syndromes, of whom eight had myocardial infarction.

### Changes of cytokine values over time and comparison with the control group

MCSF (median, 25th to 75th centile) showed a significant increase at discharge (446 pg/ml, 310 to 686) compared with admission values (373 pg/ml, 288 to 603) but there was a decline at six weeks (331 pg/ml, 250 to 464) (fig 1A).

IL-6 showed the highest values on admission (6.5 pg/ml, 3.0 to 11.4) (fig 1B); there was a decrease at discharge (5.2 pg/ml, 2.9 to 10.0) and a further decline at six weeks (5 pg/ml, 2.7 to 8.7).

TNF $\alpha$  showed a significant increase at discharge (4.9 pg/ml, 3.4 to 6.6) compared with admission values (4 pg/ml, 2.8 to 5.6) but declined at six weeks (4 pg/ml, 3.0 to 5.5) (fig 1C).

Cytokine values (pg/ml) at six weeks were significantly higher than in the control group—MCSF: 331 (250 to 464) *v* 302 (234 to 378),  $p = 0.003$ ; IL-6: 5.0 (2.7 to 8.7) *v* 2.4 (1.4 to 3.6),  $p = 0.0001$ ; and TNF $\alpha$ : 4.0 (3.0 to 5.5) *v* 3.8 (3.2 to 4.6),  $p = 0.03$ .

### Long term prognostic value of cytokines

There was a trend for higher cytokine values on admission or at discharge in patients with cardiac events than in those without events, but the difference did not reach significance (table 2). However, MCSF and IL-6 concentrations at six weeks were higher in patients with cardiac events during the two year follow up, and the difference was significant. Logistic regression analysis (dependent variable: cardiac events; independent variables: age, sex, hypertension, diabetes mellitus, MCSF, IL-6, and TNF $\alpha$ ) showed that MCSF values were the only independent predictors of future events, with an adjusted odds ratio for events of 4.1 (95% CI 1.1 to 14.8;  $p = 0.03$ ) (table 3). MCSF values at six weeks were related to IL-6 ( $r = 0.45$ ,  $p = 0.0001$ ) and TNF $\alpha$  concentrations ( $r = 0.21$ ,  $p = 0.03$ ). To elucidate whether this correlation could have contributed to the failure of IL-6 or TNF $\alpha$  concentrations to enter the regression model we repeated the analysis after removing IL-6 and TNF $\alpha$ . MCSF remained an independent predictor of events, while IL-6 and TNF $\alpha$  were not independently associated with the risk of future events even after exclusion of MCSF from the model. Finally, admission values of MCSF were related to admission

**Table 1** Clinical characteristics and lipid profile of patients with unstable angina in relation to the development of cardiac events during the two year follow up

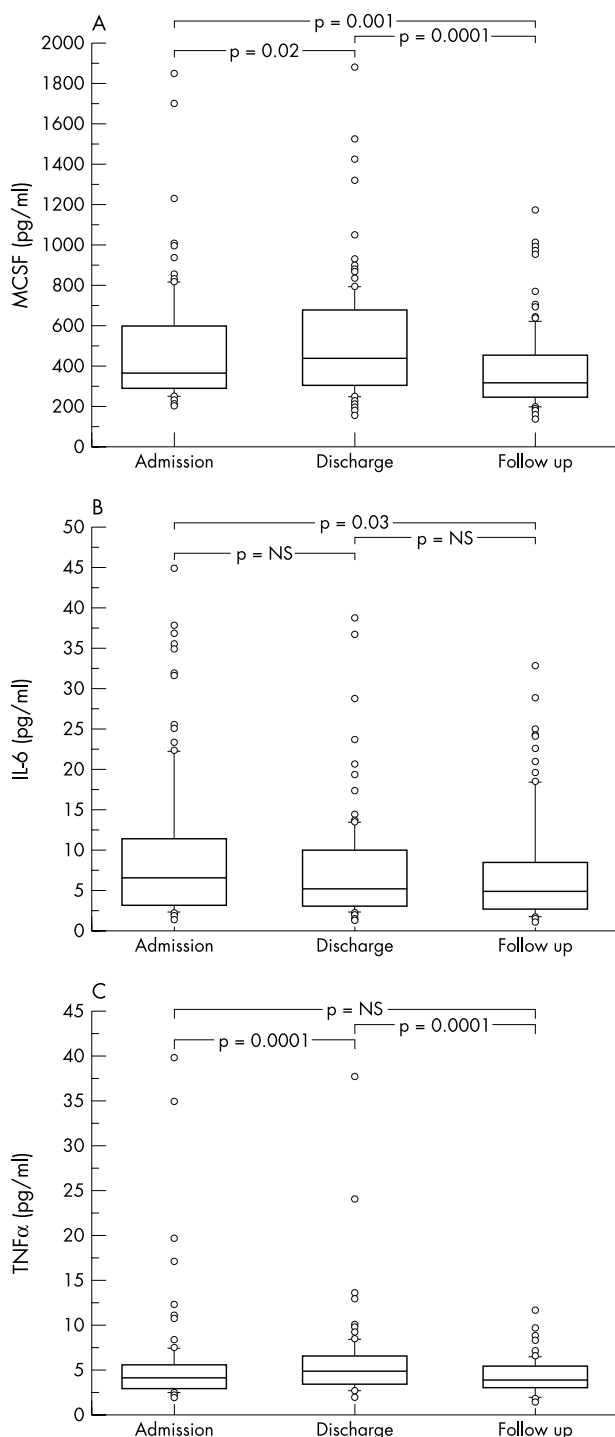
Variable	Cardiac event (n = 39)	No cardiac event (n = 72)	p Value
Age (years)	61.9 (9.1)	57 (10.5)	0.01
Sex (male/female)	29/10	59/13	0.8
Body mass index (kg/m <sup>2</sup> )	28.6 (4.7)	27.7 (3.6)	0.3
Hypertension	21/39 (53.8%)	27/72 (37.5%)	0.14
Diabetes mellitus	15/39 (38.5%)	17/72 (23.6%)	0.15
Family history of CAD	8/39 (20.5%)	13/72 (18%)	0.95
Current smokers*	21/39 (53.8%)	41/72 (56.9%)	0.9
Lipid†			
Total cholesterol (mmol/l)	5.51 (0.98)	5.55 (0.78)	0.8
Triglycerides (mmol/l)	1.73 (0.69)	1.57 (0.63)	0.3
HDL cholesterol (mmol/l)	0.96 (0.23)	1.01 (0.23)	0.3
LDL cholesterol (mmol/l)	3.7 (0.84)	3.82 (0.72)	0.4

Values are mean (SD) or n (%).

\*Smokers until admission for unstable angina.

†Lipids were measured six weeks after discharge.

CAD, coronary artery disease; HDL, high density lipoprotein; LDL, low density lipoprotein.



**Figure 1** Comparison between admission, discharge, and follow up serum concentrations at six weeks of (A) macrophage colony stimulating factor (MCSF), (B) interleukin-6 (IL-6), and (C) tumour necrosis factor  $\alpha$  (TNF $\alpha$ ). Values are expressed as median and 25th and 75th centiles by boxes, with the largest and smallest non-outlier values by the lines at the ends of the boxes, and outlier values by circles.

values of IL-6 ( $r = 0.55$ ,  $p = 0.0001$ ) and TNF $\alpha$  ( $r = 0.41$ ,  $p = 0.0001$ ), and discharge MCSF values were related to IL-6 ( $r = 0.37$ ,  $p = 0.0002$ ) and TNF $\alpha$  at discharge ( $r = 0.50$ ,  $p = 0.0001$ ).

The two year survival free from readmission, revascularisation, and cardiac death was significantly shorter in patients with MCSF values in the highest tertile (values

$\geq 468$  pg/ml) than in patients with values  $< 468$  pg/ml (fig 2).

## DISCUSSION

Our study indicates that MCSF concentrations determined six weeks after discharge in patients with severe unstable angina were strong predictors of cardiac events during a two year follow up. In contrast, admission or discharge cytokine values were not predictive of long term outcome.

### Rationale for late cytokine measurements

We propose that cytokine determination beyond the acute phase of a coronary event may have a greater long term prognostic value than measurements made during the hospital phase. This is a novel concept. Only one recent study<sup>7</sup> in patients with acute myocardial infarction has shown that measurement of an inflammatory mediator (C reactive protein) shortly after myocardial infarction was not predictive of long term cardiac events. It was suggested that for chronic cardiovascular risk assessment, measurements should be delayed at least one month beyond the hospital phase of the acute infarct.

The delayed measurement of cytokines in assessing the long term predictive value has two potential advantages. The first is that we overcome the "problem" that is posed on prognosis by the easy access to revascularisation, either during the hospital admission (urgently) or early after discharge (electively). This early invasive treatment in unstable angina may change the natural history of coronary artery disease and therefore attenuate the long term prognostic value of an inflammatory marker measured during the acute phase. A similar issue was raised previously by Toss and colleagues<sup>4</sup> who, commenting on the lack of an association of fibrinogen values with the risk of death or myocardial infarction during a 42 day follow up in a subgroup of patients with unstable angina in the TIMI (thrombolysis in myocardial infarction) IIIB trial, suggested that early coronary angioplasty could have modified the natural course of the disease. The second advantage is that six weeks after the coronary event the excess inflammatory response caused by the event has decreased to the new steady state level or "normalised", and inflammatory markers may then reflect the underlying chronic inflammatory process and be better indicators of the severity of the disease. Thus a persistent elevation of cytokines beyond the acute phase suggests prolonged inflammation and could represent a subclinical hallmark of recurrent instability.<sup>8</sup>

It is not clear when the inflammatory response is "normalised" after an acute coronary event. Ueda and colleagues showed that elevation of inflammatory markers caused by acute myocardial infarction is normalised within four weeks after onset.<sup>9</sup> However, Mulvihill and colleagues suggested that the inflammation is sustained for up to six months after the presentation of unstable angina.<sup>10</sup> In that study the inflammatory response was assessed by measuring the concentrations of soluble forms of cellular adhesion molecules. However, there were no follow up data to explore whether this prolonged inflammation was related to prognosis.

### Macrophage colony stimulating factor and long term prognosis

MCSF is a haematopoietic growth factor released by the injured endothelium, and its primary function is the regulation of proliferation, differentiation, and maturation of monocytes and macrophages.<sup>11</sup> Macrophage involvement in cardiac events includes various mechanisms such as thrombus organisation, smooth muscle cell migration and proliferation, and secretion of proteolytic enzymes.<sup>12</sup> It has

**Table 2** Macrophage colony stimulating factor (MCSF), interleukin-6 (IL-6), and tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) concentrations on admission, at discharge, and six weeks later in patients with or without cardiac events during the two year follow up

	Cardiac event (n = 39)	No cardiac event (n = 72)	p Value
<i>On admission</i>			
MCSF (pg/ml)	461 (309 to 603)	372 (281 to 601)	0.15
IL-6 (pg/ml)	7.1 (4.4 to 11.8)	4.8 (2.8 to 10.7)	0.12
TNF $\alpha$ (pg/ml)	4.7 (3.5 to 5.8)	3.7 (2.8 to 5.1)	0.10
<i>At discharge</i>			
MCSF (pg/ml)	521 (358 to 703)	417 (303 to 574)	0.06
IL-6 (pg/ml)	6 (3.0 to 9.6)	4.7 (2.95 to 10.25)	0.80
TNF $\alpha$ (pg/ml)	5.1 (3.6 to 7.0)	4.6 (3.3 to 6.3)	0.23
<i>At six weeks</i>			
MCSF (pg/ml)	424 (315 to 578)	306 (233 to 396)	0.0008
IL-6 (pg/ml)	6.6 (3.5 to 11.2)	4.5 (2.3 to 7.4)	0.01
TNF $\alpha$ (pg/ml)	4.6 (3.3 to 6.1)	4.3 (3.2 to 5.7)	0.38

Values are median (25th to 75th centile).

been proposed that MCSF may initiate and prolong ischaemic episodes by promoting the formation of microthrombi,<sup>13</sup> increasing coronary tone,<sup>14</sup> and impairing vasodilatation.<sup>15</sup>

Previous studies have shown that MCSF concentrations were higher in patients with unstable than with stable angina.<sup>5, 16, 17</sup> In addition, raised MCSF values could predict worse in-hospital prognosis in patients with unstable angina.<sup>18</sup> Very little information is available on the long term predictive value of MCSF concentrations in unstable angina. Saitoh and colleagues demonstrated that high MCSF values ( $\geq 950$  pg/ml) predicted cardiac events during a 14 month follow up in a mixed population of 97 patients with stable and 45 patients with unstable angina.<sup>5</sup> In that study MCSF concentrations were determined before discharge. In our study we recruited only patients with severe unstable angina, and cytokine values at discharge were not predictive of long term outcome. However, MCSF concentrations measured six weeks later were able to predict cardiac events independently. Our findings support the hypothesis that MCSF plays an active role in destabilising the atheromatous plaque, triggering ischaemia, and causing coronary events. Recent data have also reinforced the possibility of active participation of MCSF in plaque destabilisation. Seshiah and colleagues showed that MCSF can induce apoptosis of vascular smooth muscle cells and therefore cause plaque rupture by weakening the coronary plaque.<sup>19</sup> In another study,<sup>20</sup> MCSF caused an increase in the expression of membrane type 3 matrix metalloproteinase within human complex plaques, promoting degradation of extracellular matrix and thereby plaque destabilisation.

Interestingly, MCSF concentrations were higher at discharge than on admission, suggesting that MCSF requires a few days before it reaches its highest concentration. Unfortunately, we are unable to define the precise time

course of peaking of MCSF as we did not obtain serial measurements during the hospital admission.

Finally, the use of drugs that could have affected the long term prognosis in these patients was similar in the two subgroups. There was no difference in the use of aspirin,  $\beta$  blockers, angiotensin converting enzyme inhibitors, and statins during (data not reported) or at the end of the follow up period between patients with and without cardiac events.

### IL-6, TNF $\alpha$ , and long term prognosis

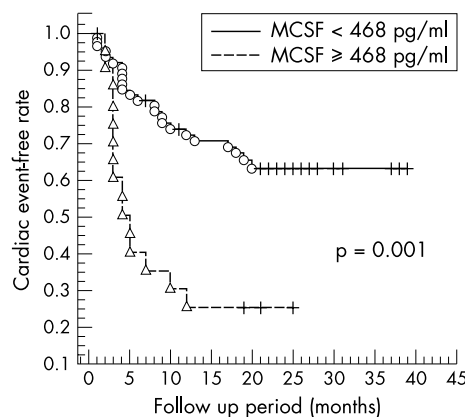
IL-6 is a pleiotropic cytokine produced in the liver which induces the synthesis of acute phase proteins such as C reactive protein and fibrinogen.<sup>21–23</sup> It has both inflammatory and procoagulant properties.<sup>9, 24</sup> Higher concentrations have been found in unstable angina, and it has been reported to predict short term prognosis.<sup>25</sup> In our study IL-6 concentrations at six weeks were also higher in patients with cardiac events than in those without events. However, IL-6 could not independently predict events—even after exclusion of MCSF from the regression model, IL-6 was not an independent predictor of events.

We found a strong positive association between MCSF and IL-6 values on admission, at discharge, and at follow up. Although the precise trigger of the inflammatory cascade in an acute coronary event is unknown, it is tempting to speculate that oxidised low density lipoprotein (LDL) is the

**Table 3** Odds ratios for cardiac events during the two year follow up period

	Odds ratio	95% CI	p Value
MCSF	4.1	1.1 to 14.8	0.03
IL-6	1.29	0.68 to 2.4	0.4
TNF $\alpha$	0.83	0.27 to 2.5	0.7
Age	1.04	0.99 to 1.1	0.1
Sex	0.7	0.21 to 2.35	0.6
Hypertension	1.7	0.62 to 4.60	0.3
Diabetes mellitus	1.34	0.47 to 3.80	0.6

CI, confidence interval; IL-6, interleukin-6; MCSF, macrophage colony stimulating factor; TNF $\alpha$ , tumour necrosis factor  $\alpha$ .

**Figure 2** Cumulative two year cardiac event-free survival curves for patients with severe unstable angina. The cardiac event-free rate in patients with macrophage colony stimulating factor (MCSF) concentrations  $\geq 468$  pg/ml was significantly lower than in patients with MCSF concentration  $< 468$  pg/ml ( $p = 0.001$ ).



common inducer of MCSF and IL-6 production. This assumption is based on experimental work which has shown that oxidised LDL stimulates the production of MCSF and IL-6 from endothelial and liver cells, respectively.<sup>26, 27</sup>

TNF $\alpha$  is a cytokine with a wide range of proinflammatory activities.<sup>28</sup> Plasma TNF $\alpha$  concentration is associated with early atherosclerosis and correlates with metabolic and cellular perturbations that are considered important for vascular events.<sup>29</sup> In addition, persistently increased concentrations of TNF $\alpha$  among patients who sustained a myocardial infarct were predictive of recurrent coronary events.<sup>30</sup> In our study we failed to show an association between TNF $\alpha$  concentrations and long term prognosis.

### Study limitations

The major limitation of the study is the relatively small size of the sample. Thus the study may not have been adequately powered to demonstrate a prognostic effect of admission and discharge MCSF values. The same may be true for IL-6 and TNF $\alpha$  concentrations. Larger studies are necessary to investigate whether the prognostic value of MCSF is independent of or additive to that of C reactive protein, a reliable, low cost, and extensively studied inflammatory marker. Finally, our study does not answer the question of what is the best time to measure MCSF concentrations for long term prognosis. We chose six weeks after the acute episode on the basis that it takes a few weeks before the inflammatory markers fall to a new steady state level. However, this choice is to an extent arbitrary, and serial measurements of MCSF concentrations for a period of 2–6 months after discharge could help determine the appropriate time more precisely.

### Conclusions

Our study suggests that increased MCSF concentrations six weeks after the acute phase can predict a worse long term prognosis in patients with severe unstable angina. It is possible that MCSF plays an important role in the progression and destabilisation of atherosclerotic lesions. In addition, our findings support the hypothesis that inflammatory markers determined beyond the acute phase are better predictors of future cardiac events in acute coronary syndromes than markers determined during the acute phase.

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